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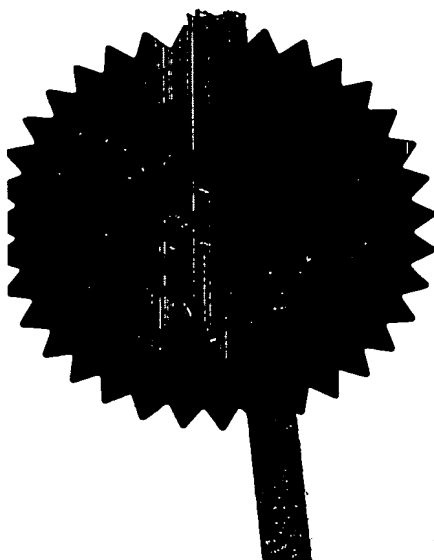
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05APR02 E708918-4 D00239
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4. Title of the invention "SCHIZOPHRENIA ASSOCIATED GENE (IV) "

5. Name of your agent (if you have one)

CRUIKSHANK & FAIRWEATHER

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SCHIZOPHRENIA ASSOCIATED GENE (IV)

The present invention relates to the identification of a gene which has been disrupted in a patient diagnosed as suffering from schizophrenia, as well as proteins encoded by the gene and antibodies thereto and to uses of such products as medicaments for treating schizophrenia and/or affective psychosis. The invention also relates to methods for diagnosing patients suffering or predisposed to schizophrenia and/or affective psychosis, as well as screens for developing novel treatment regimes for schizophrenia and/or affective psychosis.

Schizophrenia and affective psychoses such as Bipolar Affective Disorder are common and debilitating psychiatric disorders. Despite a wealth of information on the epidemiology, neuroanatomy and pharmacology of the illness, it is uncertain what molecular pathways are involved and how impairments in these affect brain development and neuronal function. Despite an estimated heritability of 60-80%, very little is known about the number or identity of genes involved in these psychoses. Although there has been recent progress in linkage and association studies, especially from genome-wide scans, these studies have yet to progress from the identification of susceptibility loci or candidate genes to the full characterisation of disease-causing genes (Berrettini, 2000).

The cloning of breakpoints in patients with chromosome abnormalities (translocations, inversions etc.) has proved instrumental in the identification of many disease genes

(e.g. Duchenne Muscular Dystrophy, Retinoblastoma, Wilm's Tumour, Familial Polyposis Coli, Fragile-X Syndrome, Polycystic Kidney Disease, many leukaemias and, very recently, a candidate speech and language disorder gene (Lai et al, 2001)). Such studies assume that the chromosomal breakpoints give rise to the clinical symptoms by either directly disrupting gene sequences or perturbing gene expression. In the latter case, it is considered that the breakpoint can still affect expression of a gene at a distance of at least up to 1Mb (Kleinjan & van Heyningen, 1998). In the same way that gene-trap mutagenesis can be used to identify disrupted mouse genes (Brennan & Skarnes, 1999), the physical "flag" created by a cytogenetic breakpoint provides a geographical pointer for the disease locus.

It is amongst the objects of the present invention to provide a gene and/or protein postulated to be involved with the development and/or symptoms associated with schizophrenia and/or affective psychosis.

The present invention is based on the characterisation of a balanced reciprocal translocation between chromosomes 9 and 14, $t(9;14)(q34;q13)$ in a mother with schizophrenia and her daughter with schizophrenia co-morbid with mild learning disability. A brain transcription factor gene, *NPAS3*, is shown to be disrupted by the translocation at 14q13. Without wishing to be bound by theory, the present inventors hypothesis is that the disruption of this gene is responsible for the psychotic symptoms exhibited by the

mother and daughter.

In a first aspect the present invention provides use of a polynucleotide fragment comprising the *NPAS3* gene or a fragment, derivative or homologue thereof for the manufacture of a medicament for treating schizophrenia and/or affective psychosis in a subject.

In another aspect the present invention provides use of a polypeptide fragment encoded by the *NPAS3* gene, or a fragment, derivative or homologue thereof for the manufacture of a medicament for treating schizophrenia and/or affective psychosis in a subject.

Schizophrenia and/or affective psychosis as used herein relates to schizophrenia, as well as other affective psychoses such as those listed in "The ICD-10 Classification of Mental and Behavioural Disorders" World Health Organization, Geneva 1992. Categories F20 to F29 inclusive includes Schizophrenia, schizotypal and delusional disorders. Categories F30 to F39 inclusive are Mood (affective) disorders that include bipolar affective disorder and depressive disorder. Mental Retardation is coded F70 to F79 inclusive. The Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DMS-IV). American Psychiatric Association, Washington DC. 1994. Include all conditions coded 295.xx (Schizophrenia and Other Psychotic Disorders) and 296.xx (Major Depressive Disorders and Bipolar Disorders). Mental Retardation is coded 315, 317, 318 and 319.

The human form of NPAS3 has previously been identified and is found in the public database under accession numbers AB054575 and AF164438, with the differences due to alternative splicing and all forms are encompassed within the present invention.

Thus, references herein to the NPAS3 gene are understood to relate to the sequences identified in Figures 1 and 3 and references to the NPAS3 protein sequence are understood to relate to the sequences identified in Figures 2 and 4.

In certain jurisdictions claims to methods of treatment are permissible and so the skilled reader will appreciate that the NPAS3 gene, or fragment, derivative or homologue thereof; or NPAS3 protein, or functionally active fragment, derivative, or homologue thereof, may be administered to an individual as a method of treating an individual with schizophrenia and/or affective psychosis.

"Polynucleotide fragment" as used herein refers to a chain of nucleotides such as deoxyribose nucleic acid (DNA) and transcription products thereof, such as RNA. Naturally, the skilled addressee will appreciate the whole naturally occurring human genome is not included in the definition of polynucleotide fragment.

The polynucleotide fragment can be isolated in the sense that it is substantially free of biological material with which the whole genome is normally associated *in vivo*. The isolated polynucleotide fragment may be cloned to provide a recombinant molecule comprising the

polynucleotide fragment. Thus, "polynucleotide fragment" includes double and single stranded DNA, RNA and polynucleotide sequences derived therefrom, for example, subsequences of said fragment and which are of any desirable length. Where a nucleic acid is single stranded then both a given strand and a sequence or reverse complementary thereto is within the scope of the present invention.

In general, the term "expression product" or "gene product" refers to both transcription and translation products of said polynucleotide fragments. When the expression or gene product is a "polypeptide" (i.e. a chain or sequence of amino acids displaying a biological activity substantially similar (eg. 98%, 95%, 90%, 80%, 75% activity) to the biological activity of the protein), it does not refer to a specific length of the product as such. Thus, the skilled addressee will appreciate that "polypeptide" encompasses *inter alia* peptides, polypeptides and proteins. The polypeptide if required, can be modified *in vivo* and *in vitro*, for example by glycosylation, amidation, carboxylation, phosphorylation and/or post-translational cleavage.

The present invention further provides a recombinant or synthetic polypeptide for the manufacture of reagents for use as therapeutic agents in the treatment of schizophrenia and/or affective psychosis. In particular, the invention provides pharmaceutical compositions comprising the recombinant or synthetic polypeptide

together with a pharmaceutically acceptable carrier therefor.

The present invention further provides an isolated polynucleotide fragment capable of specifically hybridising to a related polynucleotide sequence from another species. In this manner, the present invention provides probes and/or primers for use in *ex vivo* and/or *in situ* detection and expression studies. Typical detection studies include polymerase chain reaction (PCR) studies, hybridisation studies, or sequencing studies. In principle any specific polynucleotide sequence fragment from the identified sequences may be used in detection and/or expression studies. The skilled addressee understands that a specific fragment is a fragment of the sequence which is of sufficient length, generally greater than 10, 12, 14, 16 or 20 nucleotides in length, to bind specifically to the sequence, under conditions of high stringency, as defined herein, and not bind to unrelated sequences, that is sequences from elsewhere in the genome of the organism other than an allelic form of the sequence or non-homologous sequences from other organisms.

"Capable of specifically hybridising" is taken to mean that said polynucleotide fragment preferably hybridises to a related or similar polynucleotide sequence in preference to unrelated or dissimilar polynucleotide sequences.

The invention includes polynucleotide sequence(s) which are capable of specifically hybridising to an polynucleotide fragment as described herein or to a part

thereof without necessarily being completely complementary or reverse complementary to said related polynucleotide sequence or fragment thereof. For example, there may be at least 50%, or at least 75%, at least 90%, or at least 95% complementarity. Of course, in some cases the sequences may be exactly reverse complementary (100% reverse complementary) or nearly so (e.g. there may be less than 10, typically less than 5 mismatches). Thus, the present invention also provides anti-sense or complementary nucleotide sequence(s) which is/are capable of specifically hybridising to the disclosed polynucleotide sequence. If a specific polynucleotide is to be used as a primer in PCR and/or sequencing studies, the polynucleotide must be capable of hybridising to related nucleic acid and capable of initiating chain extension from 3' end of the polynucleotide, but not able to correctly initiate chain extension from unrelated sequences.

If a polynucleotide sequence of the present invention is to be used in hybridisation studies to obtain or identify a related sequence from another organism the polynucleotide sequence should preferably remain hybridised to a sample polynucleotide under stringent conditions. If desired, either the test or sample polynucleotide may be immobilised. Generally the test polynucleotide sequence is at least 10, 14, 20 or at least 50 bases in length. It may be labelled by suitable techniques known in the art. Preferably the test polynucleotide sequence is at least 200 bases in length and may even be several kilobases in

length. Thus, either a denatured sample or test sequence can be first bound to a support. Hybridization can be effected at a temperature of between 50 and 70°C in double strength SSC (2xNaCl 17.5g/l and sodium citrate (SC) at 8.8g/l) buffered saline containing 0.1% sodium dodecyl sulphate (SDS). This can be followed by rinsing of the support at the same temperature but with a buffer having a reduced SSC concentration. Depending upon the degree of stringency required, and thus the degree of similarity of the sequences, such reduced concentration buffers are typically single strength SSC containing 0.1%SDS, half strength SSC containing 0.1%SDS and one tenth strength SSC containing 0.1%SDS. Sequences having the greatest degree of similarity are those the hybridisation of which is least affected by washing in buffers of reduced concentration. It is most preferred that the sample and inventive sequences are so similar that the hybridisation between them is substantially unaffected by washing or incubation in standard sodium citrate (0.1 x SSC) buffer containing 0.1%SDS.

Oligonucleotides may be designed to specifically hybridise to NPAS3 nucleic acid. They may be synthesised, by known techniques and used as primers in PCR or sequencing reactions or as probes in hybridisations designed to detect the presence of mutated or normal NPAS3 in a sample. The oligonucleotides may be labelled by suitable labels known in the art, such as, radioactive labels, chemiluminescent labels or fluorescent labels and

the like.

The term "oligonucleotide" is not meant to indicate any particular length of sequence and encompasses nucleotides of preferably at least 10b (e.g. 10b to 1kb) in length, more preferably 12b-500b in length and most preferably 15b to 100b.

The oligonucleotides may be designed with respect to any of the sequences described herein and may be manufactured according to known techniques. They may have substantial sequence identity (e.g. at least 50%, at least 75%, at least 90% or at least 95% sequence identity) with one of the strands shown therein or an RNA equivalent, or with a part of such a strand. Preferably such a part is at least 10, at least 30, at least 50 or at least 200 bases long. It may be an open reading frame (ORF) or a part thereof.

Oligonucleotides which are generally greater than 30 bases in length should preferably remain hybridised to a sample polynucleotide under one or more of the stringent conditions mentioned above. Oligonucleotides which are generally less than 30 bases in length should also preferably remain hybridised to a sample polynucleotide but under different conditions of high stringency. Typically the melting temperature of an oligonucleotide less than 30 bases may be calculated according to the formula of; 2°C for every A or T, plus 4°C for every G or C, minus 5°C . Hybridization may take place at or around the calculated melting temperature for any particular oligonucleotide, in

6 x SSC and 1% SDS. Non specifically hybridised oligonucleotides may then be removed by stringent washing, for example in 3 x SSC and 0.1% SDS at the same temperature. Only substantially similar matched sequences remain hybridised i.e. said oligonucleotide and corresponding test nucleic acid.

When oligonucleotides of generally less than 30 bases in length are used in sequencing and/or PCR studies, the melting temperature may be calculated in the same manner as described above. The oligonucleotide may then be allowed to anneal or hybridise at a temperature around the oligonucleotides calculated melting temperature. In the case of PCR studies the annealing temperature should be around the lower of the calculated melting temperatures for the two priming oligonucleotides. It is to be appreciated that the conditions and melting temperature calculations are provided by way of example only and are not intended to be limiting. It is possible through the experience of the experimenter to vary the conditions of hybridisation and thus anneal/hybridise oligonucleotides at temperatures above their calculated melting temperature. Indeed this can be desirable in preventing so-called non-specific hybridisation from occurring.

It is possible when conducting PCR studies to predict an expected size or sizes of PCR product(s) obtainable using an appropriate combination of two or more oligonucleotides, based on where they would hybridise to the sequences described herein. If, on conducting such a

PCR on a sample of DNA, a fragment of the predicted size is obtained, then this is predictive that the DNA encodes a homologous sequence from a test organism.

Proteins for all the applications described herein can be produced by cloning the gene for example into plasmid vectors that allow high expression in a system of choice e.g. insect cell culture, yeast, animal cells, bacteria such as *Escherichia coli*. To enable effective purification of the protein, a vector may be used that incorporates an epitope tag (or other "sticky" extension such as His6) onto the protein on synthesis. A number of such vectors and purification systems are commercially available.

The polynucleotide fragment can be molecularly cloned into a prokaryotic or eukaryotic expression vector using standard techniques and administered to a host. The expression vector is taken up by cells and the polynucleotide fragment of interest expressed, producing protein.

It will be understood that for the particular polypeptides embraced herein, natural variations such as may occur due to polymorphisms, can exist between individuals or between members of the family. These variations may be demonstrated by (an) amino acid difference(s) in the overall sequence or by deletions, substitutions, insertions, inversions or additions of (an) amino acid(s) in said sequence. All such derivatives showing the recognised activity are included within the scope of the invention. For example, for the purpose of

the present invention conservative replacements may be made between amino acids within the following groups:

- (I) Alanine, serine, threonine;
- (II) Glutamic acid and aspartic acid;
- (III) Arginine and leucine;
- (IV) Asparagine and glutamine;
- (V) Isoleucine, leucine and valine;
- (VI) Phenylalanine, tyrosine and tryptophan

Moreover, recombinant DNA technology may be used to prepare nucleic acid sequences encoding the various derivatives outlined above.

As is well known in the art, the degeneracy of the genetic code permits substitution of bases in a codon resulting in a different codon which is still capable of coding for the same amino acid, e.g. the codon for amino acid glutamic acid is both GAT and GAA. Consequently, it is clear that for the expression of polypeptides from nucleotide sequences described herein or fragments thereof, use can be made of a derivative nucleic acid sequence with such an alternative codon composition different from the nucleic acid sequences shown in the Figures.

The polynucleotide fragments of the present invention are preferably linked to regulatory control sequences. Such control sequences may comprise promoters, operators, inducers, enhancers, silencers, ribosome binding sites, terminators etc. Suitable control sequences for a given host may be selected by those of ordinary skill in the art.

A polynucleotide fragment according to the present invention can be ligated to various expression controlling sequences, resulting in a so called recombinant nucleic acid molecule. Thus, the present invention also includes an expression vector containing an expressible nucleic acid molecule. The recombinant nucleic acid molecule can then be used for the transformation of a suitable host.

Specific vectors which can be used to clone nucleic acid sequences according to the invention are known in the art (e.g. Rodriguez, R.L. and Denhardt, D.T., Edit., Vectors: a survey of molecular cloning vectors and their uses, Butterworths, 1988, or Jones et al., Vectors: Cloning Applications: Essential Techniques (Essential techniques series), John Wiley & Son. 1998).

The methods to be used for the construction of a recombinant nucleic acid molecule according to the invention are known to those of ordinary skill in the art and are inter alia set forth in Sambrook, et al. (Molecular Cloning: a laboratory manual Cold Spring Harbour Laboratory, 1989).

The present invention also relates to a transformed cell containing the polynucleotide fragment in an expressible form. "Transformation", as used herein, refers to the introduction of a heterologous polynucleotide fragment into a host cell. The method used may be any known in the art, for example, direct uptake, transfection transduction or electroporation (Current Protocols in Molecular Biology, 1995. John Wiley and Sons Inc.). The

heterologous polynucleotide fragment may be maintained through autonomous replication or alternatively, may be integrated into the host genome. The recombinant nucleic acid molecules preferably are provided with appropriate control sequences compatible with the designated host which can regulate the expression of the inserted polynucleotide fragment, e.g. tetracycline responsive promoter, thymidine kinase promoter, SV-40 promoter and the like.

Suitable hosts for the expression of recombinant nucleic acid molecules may be prokaryotic or eukaryotic in origin. Hosts suitable for the expression of recombinant nucleic acid molecules may be selected from bacteria, yeast, insect cells and mammalian cells.

In another aspect the present invention also relates to a method of diagnosing schizophrenia and/or affective psychosis or susceptibility to schizophrenia and/or affective psychosis in an individual, wherein the method comprises determining if the *NPAS3* gene in the individual has been disrupted by a mutation or chromosomal rearrangement.

The methods which may be employed to elucidate such a mutation or chromosomal rearrangement are well known to those of skill in the art and could be detected for example using PCR or in hybridisation studies using suitable probes which could be designed to span an identified mutation site or in the *NPAS3* gene, such as the breakpoint identified by the present inventors and described herein.

Once a particular polymorphism or mutation has been identified it may be possible to determine a particular course of treatment. For example it is known that some forms of treatment work for some patients, but not all. This may in fact be due to mutations in the *NPAS3* gene or surrounding sequence, and it may therefore be possible to determine a treatment strategy using current therapies, based on a patient's genotype.

It will be appreciated that mutations in the gene sequence or controlling elements of a gene, eg. a promoter and/or enhance can have subtle effects such as affecting mRNA splicing/stability/activity and/or control of gene expression levels, which can also be determined.

Also the relative levels of RNA can be determined using for example hybridisation or quantitative PCR as a means to determine if the *NPAS3* gene has been disrupted.

Moreover the presence and/or levels of the *NPAS3* gene products themselves can be assayed by immunological techniques such as radioimmunoassay, Western blotting and ELISA using specific antibodies raised against the gene products. The present invention also therefore relates to antibodies specific for a *NPAS3* gene product and uses thereof in diagnosis and/or therapy.

A further aspect of the present invention therefore provides antibodies specific to the polypeptides of the present invention or epitopes thereof. Production and purification of antibodies specific to an antigen is a matter of ordinary skill, and the methods to be used are

clear to those skilled in the art. The term antibodies can include, but is not limited to polyclonal antibodies, monoclonal antibodies (mAbs), humanised or chimeric antibodies, single chain antibodies, Fab fragments, F(ab')₂ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope binding fragments of any of the above. Such antibodies may be used in modulating the expression or activity of the particular polypeptide, or in detecting said polypeptide *in vivo* or *in vitro*.

Using the sequences disclosed herein, it is possible to identify related sequences in other animals, such as mammals, with the intention of providing an animal model for psychiatric disorders associated with the improper functioning of the nucleotide sequences and proteins of the present invention. Once identified, the homologous sequences can be manipulated in several ways common to the skilled person in order to alter the functionality of the nucleotide sequences and proteins homologous to those of the present invention. For example, "knock-out" animals may be created, that is, the expression of the genes comprising the nucleotide sequences homologous to those of the present invention may be reduced or substantially eliminated in order to determine the effects of reducing or substantially eliminating the expression of such genes. Alternatively, animals may be created where the expression of the nucleotide sequences and proteins homologous to those of the present invention are upregulated, that is,

the expression of the genes comprising the nucleotide sequences homologous to those of the present invention may be increased in order to determine the effects of increasing the expression of these genes. In addition to these manipulations, substitutions, deletions and additions may be made to the nucleotide sequences encoding the proteins homologous to those of the present invention in order to effect changes in the activity of the proteins to help elucidate the function of domains, amino acids, etc. in the proteins. Furthermore, the sequences of the present invention may also be used to transform animals to the manner described above. The manipulations described above may also be used to create an animal model of schizophrenia and/or affective psychosis associated with the improper functioning of the nucleotide sequences and/or proteins of the present invention in order to evaluate potential agents which may be effective for combatting psychotic disorders, such as schizophrenia and/or affective psychosis.

Thus, the present invention also provides for screens for identifying agents suitable for preventing and/or treating schizophrenia and/or affective psychosis associated with disruption or alteration in the expression of the *NPAS3* gene and/or its gene products. Such screens may easily be adapted to be used for the high throughput screening of libraries of compounds such as synthetic, natural or combinatorial compound libraries.

Thus, the *NPAS3* gene products according to the present invention can be used for the *in vivo* or *in vitro* identification of novel ligands or analogs thereof. For this purpose binding studies can be performed with cells transformed with nucleotide fragments according to the invention or an expression vector comprising a polynucleotide fragment according to the invention, said cells expressing the *NPAS3* gene products according to the invention.

Alternatively also the *NPAS3* gene products according to the invention as well as ligand-binding domains thereof can be used in an assay for the identification of functional ligands or analogs for the *NPAS3* gene products.

Methods to determine binding to expressed gene products as well as *in vitro* and *in vivo* assays to determine biological activity of gene products are well known. In general, expressed gene product is contacted with the compound to be tested and binding, stimulation or inhibition of a functional response is measured.

Thus, the present invention provides for a method for identifying ligands for *NPAS3* gene products, said method comprising the steps of:

- a) introducing into a suitable host cell a polynucleotide fragment according to the invention;
- b) culturing cells under conditions to allow expression of the polynucleotide fragment;
- c) optionally isolating the expression product;

d) bringing the expression product (or the host cell from step b)) into contact with potential ligands which will possibly bind to the protein encoded by said polynucleotide fragment from step a);

e) establishing whether a ligand has bound to the expressed protein; and

f) optionally isolating and identifying the ligand.

As a preferred way of detecting the binding of the ligand to the expressed protein, also signal transduction capacity may be measured.

Compounds which activate or inhibit the function of NPAS3 gene products may be employed in therapeutic treatments to activate or inhibit the polypeptides of the present invention.

The present invention will now be further described by way of Example and with reference to the Figures which show:

Table 1 probes used in FISH

Figure 1 shows the nucleic acid sequence of NPAS3 spliceform 1;

Figure 2 shows the protein sequence of NPAS3 spliceform;

Figure 3 shows the nucleic acid sequence of NPAS3 spliceform 2;

Figure 4 shows the protein sequence of NPAS3 spliceform;

Figure 5 shows an ideogram representation of the balanced translocation in the patient relating to this invention.

Figure 6 shows the genomic arrangement of the NPAS3 gene including the position of the observed breakpoint; and

Figure 7 shows potential functional consequences of the disruption to NPAS3 gene : dominant-negative activity.

Lymphocyte extraction and metaphase chromosome preparation

Lymphocytes were extracted from 7mls of patient blood (for storage and generation of EBV-transformed cell lines) using density gradient separation (Histopaque-1077, Sigma). In order to generate metaphase-arrested chromosomes for cytogenetic analysis, 0.8mls of patient blood were cultured for 71hrs in medium containing phytohaemagglutinin (Peripheral Blood Medium, Sigma). The short-term cultures were treated with colcemid for one hour followed by a conventional fixing procedure. Fixed chromosomes were dropped onto microscope slides and stored for 1 week prior to use in FISH experiments.

Selection of YAC clones for FISH probe synthesis

YAC clones were selected from the Whitehead/MIT maps of the cytogenetic interval within which the 14q12 breakpoint was adjudged to lie; accessed through the Genome Database (<http://gdbwww.gdb.org/>). YACs were obtained from the HGMP Resource Centre, Hinxton, Cambridge, UK. DNA was prepared from these clones by standard methods and

amplified by PCR using primers designed against the consensus sequence elements within the archetypal Alu repeat (Breen et al, 1992). This "Alu-PCR" gives a representative spread of non-repetitive sequence over the full length of the YAC and yields a better FISH probe synthesis than native YAC DNA. Alu-PCR was performed using the expand long template PCR kit (Roche).

Fluorescence in situ hybridisation (FISH) protocol

Pooled Alu-PCR/purified BAC or cosmid DNA was labelled with digoxigenin or biotin by nick translation and hybridised to metaphase spreads using standard FISH methods. Slides were counterstained with DAPI in Vectashield antifade solution (Vector laboratories). A Zeiss Axioskop fluorescence microscope with a chroma number 81000 multi spectral filter set was used to examine slides. Images were captured using Vysis smart capture extension running within IP Lab spectrum. The probe signal hybridised to the chromosomal region of interest indicating whether the probe was centromeric or telomeric to the breakpoint. This result dictated the choice of further DNA probes for FISH. The process continued until a probe signal was split into three distinct signals (on normal 14, der14 and der 9), indicating that it spanned the breakpoint.

Translation of YAC FISH results onto the BAC map

Markers present within the YAC were identified from the Whitehead Institute map. These were placed within a BAC contig on the UCSC GoldenPath Draft Genome Browser (<http://genome.ucsc.edu/goldenPath/hgTracks.html>) database and BAC clone order was verified by consultation of the Washington University FPC database (<http://www.genome.wustl.edu/gsc/human/Mapping/index.shtml>). RPCI-11 library BAC clones were ordered from BACPAC Resources, Children's Hospital, Oakland, Cal., USA and subsequently taken through FISH analysis as above.

Fine FISH mapping of the breakpoint with cosmid clones

PCR products corresponding to regions in or near hnPAS3 exons 4, 5 and 6 were obtained using the following primers under standard PCR conditions (Exon 4-*i* ACAACCATCTCTGGGAACAGC, Exon 4-*ii* GTGTAGGGAAAGCCATCCAA, Exon 5-*i* TCTTTTTCCTGCAGTCCCTG, Exon 5-*ii* CTCCAAATGACTCCTGCCAT, Exon 6-*i* GCCTCTGCCATAGATTTTGC, Exon 6-*ii* TTCCTTCCCACCCTTTCTCT). Probes were created by random-primed labelling of PCR products with radioactive dCTP; these were used to screen a LANL chromosome 14-specific cosmid library (LA14NC01 obtained from the UK HGMP Resource Centre, Hinxton, Cambridge) using hybridizing conditions set out in Church and Gilbert (1986). Positive clones (exon; LA1431-G5, exon 5: LA14123 - C4 and exon 6; LA1487 - D9) were prepared by a standard alkaline lysis protocol and taken through FISH analysis as above.

Results

Metaphase spreads from EBV-transformed cell lines were analysed by Fluorescence in situ Hybridisation (FISH) using successively smaller DNA probes (Fig.1). A breakpoint spanning BAC clone was obtained by FISH screening (RPCI-11 BAC 1078i14, acc. no. AL161851). EST sequences were examined in the genomic DNA flanking the breakpoint in order to identify potential transcripts in the locality. A number of ESTs were identified which had been annotated as containing homologous sequence to the conserved "PAS" domain present in a large number of genes (Gu et al, 2000). A search of such genes revealed that the most closely related gene encoded a mouse brain-expressed transcript, neuronal pas domain protein 3 (NPAS3 (MOP6), acc. no. AF137871; hereafter referred to as mNPAS3). Nucleotide homology to the mNPAS3 cDNA within human genomic DNA BAC clone sequences at 14q13 using the BLAST algorithm identified 12 exons corresponding to the human orthologue of mNPAS3 (hNPAS3) distributed over a genomic region of approximately 800-900Kb making it among the largest gene loci in the human genome (Figure 6). Subsequently, full length hNPAS3 cDNA sequences have been submitted by two other groups to GenBank/EMBL with the accession numbers, AB054575 and AF164438, although these have differences to the mouse splice-form in the 5' exons. This is due to the presence of two alternative transcription start sites employed in both human and mouse genes. This was confirmed by analysis of published cDNA and EST sequences coupled

with further sequencing of corresponding IMAGE clones. These splice variants are highlighted in Figures 1, 3 and 6.

The ratio of fluorescent signals on the derived chromosomes 9 and 14 from the breakpoint-spanning BAC probe, 1078i14, indicated that the breakpoint was located at the centromeric end of the BAC. This is the location of exon 5 of the gene. Exon 4-, 5- and 6-containing cosmids were isolated and used as FISH probes to provide definitive proof of the location of the breakpoint and confirmation that a full-length transcript (and hence protein) cannot be synthesized on the derived chromosome 14. An exon 5-containing cosmid (see Figure 6) spanned the breakpoint. Subsequently a long-range PCR product-derived FISH probe corresponding to exon 5 indicated that the breakpoint lay upstream of exon 5.

Long-range PCR primers - NPAS3 exon 5

- a) ccagcttgatatgtggtgtgg
- b) ttactcccagtgcccattgt.

Discussion

A FISH-based approach has shown that the gene, *NPAS3*, is disrupted by a chromosomal rearrangement present in a mother and daughter who suffer from comorbid schizophrenia and learning disability respectively. *NPAS3* is a brain expressed transcription factor of the basic helix-loop-helix PAS domain class which includes members such as AHR

and ARNT.

Neuronal pas3 (NPAS3) was originally cloned in the mouse (Brunskill et al, 1999) on the basis of its sequence homology with other PAS domain proteins. Its expression has been characterised in the developing mouse embryo where high levels are seen in the neural tube, neuroepithelium and, later, the neopallial layer of the cortex. Non-neural expression was also observed in the heart, limb and kidney. In the mouse, NPAS1 (human chromosomal location, 19q13) is expressed in deep pyramidal cortex cells, hippocampus and amygdala (Zhou et al., 1997). NPAS2 (human chromosomal location, 2q13) is expressed in the cortex, hippocampus and thalamus. Lower levels were also seen in spinal cord, intestines and uterus. NPAS2 was also recently deleted in mice by homologous recombination (Garcia et al., 2000) leading to deficits in cued and contextual memory. In addition NPAS2 appears to have a role in cellular energy state monitoring and the circadian rhythm pathway (Reick et al, 2001 and Rutter et al, 2001). The translocation event described herein disrupts the gene between exons 4 and 5. If transcription occurred at this disrupted locus, a truncated protein would result containing only the bHLH domain. It is conceivable that this protein would have a dominant negative effect on wild-type NPAS3 protein (or any other heterodimeric protein partner) through the creation of non-functional dimers (see Figure 7 for explanatory diagram). This would result in a potentially more severe or penetrant phenotype than a conventional point mutation.

Two examples where bHLH-PAS proteins have been altered through loss of the C-terminal PAS domain (one experimentally, the other in a patient with a chromosome translocation) have resulted in probable dominant negative action (Maemura et al, 1999, Holder jr. et al, 2000).

Mutations in this gene in karyotypically normal individuals would not be expected to have as severe or penetrant effects as those observed in the two t(9;14) patients.

Sequence comparison between hNPAS3 and other members of the NPAS sub-family show that homologies are largely restricted to the N-terminal end of the protein; the location of a basic helix-loop-helix and PAS domains. The greatest homology is with NPAS1, then NPAS2 and other PAS domain-containing proteins (data not shown). An alignment of the cognate human (conceptually translated from the splice-form containing exons 1-12) and mouse NPAS3 proteins reveals near identity over the N-terminal half of the protein but increased divergence at the C-terminal end. This is particularly the case for two stretches where 5 and 7 amino acids, respectively, have been gained in the human orthologue (Fig.4). These correspond to two poly-glycine tracts present within exon 12 (of 11 and 10 residues respectively). Such tracts can be indicative of slipped strand mispairing whereby trinucleotide repeats are aberrantly expanded or deleted. Where they occur in coding sequence, increases in the number of trinucleotide repeats can have a pathological effect on protein function (e.g.

Huntington disease and Spino-cerebellar ataxia 1). Another feature of such repeats is their unstable nature between generations: a lowering of the age of onset of a disease from generation to generation (anticipation) can often be directly linked to an increase in the number of repeat units.

Exon 12 (coding for the C-terminus of the protein) is also noteworthy because of the extremely high density of CpG dinucleotides (in humans and mouse); a feature that abruptly ends at the junctions with flanking intronic/3' sequences. This "CpG island" is unusual because it is both transcribed and also located at the 3' rather than 5' end of the gene. The significance of this in terms of potential transcriptional control by methylation or susceptibility to mutation is as yet unknown. However, the high level of G and C bases creates a bias in amino acid composition such that alanine, glycine, histidine and proline are over-represented. This may explain the presence and expansion of the poly-glycine tracts in Npas3.

14q13 is also the site of linkage to Fahr's syndrome (idiopathic basal ganglia calcification; IBGC) as determined from analysis of families (Geschwind et al, 1999). Fahr's syndrome symptoms are often accompanied by psychoses such as schizophrenia. Thus, it may be the case that NPAS3 is also the gene responsible for Fahr's syndrome.

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Figure 1

NPAS3 (NM_022123) nucleic acid sequence (spliceform 1b-3-4etc)

```
1  ccacgcgtcc gacgcccccc acccgggagg ggggagagag gcaaaaagta agagaggaaa
61  aaaaatagca ggaagatggc gccaccaag cccagcttcc agcaggatcc ttccaggcga
121  gaacgtttac aagcattgag aaaggagaaa tcccgagatg ctgctcgctc ccgccgggga
181  aaagaaaact ttgagttcta tgaattggcc aagtgtgtgc ctcttcctgc agccattacc
241  agccagctcg acaaggcatc catcattcga cttacaatta gctatctgaa aatgaggggac
301  tttgctaacc agggggaccc tccgtggaac ttgcgaatgg aaggccctcc acctaacaca
361  tcagtaaaag gtgcacagcg aaggagaagc cccagtgcac tagccattga agtatttgaa
421  gcacatttgg gaagccacat tttgcagtcc ctggatggct ttgtatttgc actaaatcag
481  gaaggaaaaa ttttgtacat ttccgaaaac gtctccatct acctaggcct ctcacaagtg
541  gagctgacag gcagcagtggt ctttgactat gtccaccccg gagatcacgt ggagatggct
601  gagcagctgg gcatgaagct cccccctggg cggggtctcc tgtcacaggg cactgctgag
661  gacggagcca gctcagcatc ttcctcctct cagtcggaga ccccgagcc agtggagtcg
721  accagcccca gtctgctaac cactgacaac actcttgagc gttccttttt catccgaatg
781  aaatctactc tgaccaaacg cgggtgtcac atcaaactat caggatataa ggtgattcac
841  ataacaggcc ggctacgcct gagagtgtcg ctgtcccacg ggaggaccgt ccccagccaa
901  atcatgggtc tcgtggttgt tgcgcagtcg ttgcctcccc ctacgatcaa tgaagtccga
961  attgactgcc atagtgtcgt cactcgagta aatatggacc tcaatatcat ttactgtgaa
1021 aataggatta gtgattatat ggatctgacc cctgtagata tcgtagggaa gagatgctac
1081 cacttcatcc atgctgaaga cgtggagggc atcaggcaca gtcacttgga cttgctgaat
1141 aagggtcagt gtgtgacaaa gtactatcgc tggatgcaga agaacggagg atatatattg
1201 atacagtcca gtgccaccat agctattaat gccagaatg caaatgaaaa gaatatcatc
1261 tgggtgaatt accttcttag caatcctgag tacaaggaca caccatgga catcgacag
1321 ctccccatc tgccggagaa aacttccgaa tcctcgga ccatccgactc tgagtccagc
1381 tctaaagaca cctcagggtat tacagaggac aacgagaact ccaagtccga cgagaagggg
1441 aaccagtccg agaacagcga agacccgagg cccgaccgga agaagtccgg caacgcgtgt
1501 gacaacgaca tgaactgcaa cgacgacggc cacagctcca gtaaccgga cagccgcgac
1561 agcgacgaca gcttcgagca ctcggaactt gagaaaccca aggcgggagg ggagggcttc
1621 ggtgctctgg gcgcgatgca gatcaagggt gagcgctacg tggagagcga gtcggacctg
1681 cggtcgaga actcgagtc actcacgtcc gacagcgcca aggactcgga cagcgagggc
1741 gaggcgggcg cgagcgctc cagcaagcac cagaagcgca agaaaaggcg gaaacggcaa
1801 aaggcgggca gcgccagccg ccggcgctcg tccagcgctg cgagccagg cggcctggac
1861 cgggcgcttg tggagccccg gcggctgctg tcctccccc acagtgcctc ggtgctcaag
1921 atcaagacgg agatctcaga acccatcaat ttcgacaatg acagcagcat ctggaactac
1981 ccgcccacc gggagatctc caggaacgag tccccctaca gcatgaccaa gcccccagc
2041 tctgagcact tcccgtcccc gcaggcgagg ggcgggtggg gtggcggtgg gggggggctg
2101 cactgtggca ttcccgaact ggtcctcacc ccgcccggcg ccgacggcgg gcccggccgc
2161 aagactcagt tcggcgccct ggccaccgag gccctggccc ccgtcgccct cgacccgctg
2221 tcacccccgc tctcggcgct ccccggggac aagcaccocg ggaacggcgg cggggcgggg
2281 ggcggggggc gcggcgcggg gggcgcgggc cccagcgctg ccaactcctt gctgtacact
2341 ggggacctgg aggcgctgca gaggttgca gggggcaacg tcgtgctccc gctggtgcac
2401 aggggtgacc ggaccctggc cgccaccagc acggccgcgc agaggggtct caccacgggc
2461 accatccgct acgcgcccgc cgaggtgacc ctggccatgc agagcaacct gctgcccac
2521 gcgcacgctg ttaacttcgt ggacgttaac agccccggt ttggcctcga cccaagacg
2581 cccatggaga tgctctacca ccacgtgcac cggtcaaca tgtcaggacc gttcggcggc
2641 gcagtgaagc cagctagcct gacgcagatg cccgcgggca acgtgttcac cacggccgag
2701 ggactcttct ccacgtgcc cttccccgtc tacagcaacg gcatccacgc ggcacgact
2761 ctggagcgca aggaggactg aggcgcggcc cgtcctgggc ccggccaggc cccgcttgga
2821 ggaggcatcg tcggcatttt cgttttagacc tttaattcta gcactttgaa ttcgagcagg
2881 tcagcgtctt ctctcgccac gacgggtccc attccacccc ctctttcttt cacctgactt
2941 attctttcgt gtaaaagatat gtttattttt tgccttcaga gggtcagacg accagttgcc
3001 tgccgttttg tcttcttcta aggtgtgtgt tgggttggtt tgctttcctt tgcatcttta
3061 ttaagatgtc tttcatgtgt atatgcctct gccatagaat actcagctct gtggtcaaga
3121 gagttctcaa gtgacaacca ttgggggttt ttcataaaga tcttgatatg atcaagatgg
3181 aaagagacaa gcataaaca tgtgccctgt ttgactaagt caaatgaaat aggggtggtt
3241 ttgtttctgt tcctaattcc ttttaaaaaa aggggaata gtattttaga attttatgca
3301 gaatttaatt ctctttttac ggtaagattt ttaagatttt cttacttgca cataaaaaa
3361 atttgggttc ttaaacttaa tttctggcct gtgactagaa tgtttaaaaa aaaaaaaac
3421 cctcgtgc
```

Figure 2

NPAS3 protein sequence (spliceform 1b-3-4etc.)

~~YAPAEVTLAMQSNLLPNAHAVNFVDVNSPGFGLDPKTPMEMLYHHVHRLNMSGPFEGG~~ QALRKEKSRDAARSRRGKENFEFYELAKLLPLPAAITSQ
LDKASIIRLTISYLKMRDFANQGDPPWNLRMEGPPPNTSVKGAQRRRSPSALAIEVF
EAHLGSHILQSLDGFVFALNQEGKFLYISETVSIYLGLSQVELTGSSVFDYVHPGDH
VEMAEQLGMKLPPGRGLLSQGTAE DGASSASSSSQSETPEPVESTSPSLLTTDNTLE
RSFFIRMKSTLTGRGVHIKSSGYKVIHITGRLRLRVSLSHGRTVPSQIMGLVVVAHA
LPPPTINEVRIDCHMFVTRVNMDLNIIYCENRISDYMDLTPVDIVGKRCYHFIHAED
VEGIRHSHLDLLNKGQCVTKYYRWMQKNGGYIWIQSSATIAINAKNANEKNI IWVNY
LLSNPEYKDT PMDIAQLPHLPEKTSSESSETSDSESDSKDTSGITEDNENSKSDEKGN
QSENSEDPEPDRKKSGNACDNDMNCNDDGHSSSNPDSRDSDDSFHSDFENPKAGED
GFGALGAMQIKVERYVESESDLRLQNCESLTSDSAKSDSAGEAGAQAASSKHQKRKK
RRKRQKGGASARRRLSSASSPGGLDAGLVEPPRLLSPPNSASVLKIKTEISEPINFD
NDSSIWNYPNREISRNE SPYSMTKPPSSEHFPS PQGGGGGGGGGGGLHVAIPDSVL
TPPGADGAAARKTQFGASATAALAPVASDPLSPPLSASPRDKHPGNGGGGGGGGGGA
GGGGPSASNSLLYTGDLEALQRLQAGNVVLPLVHRVTGTLAATSTAAQRVYTTGTIR
YAPAEVTLAMQSNLLPNAHAVNFVDVNSPGFGLDPKTPMEMLYHHVHRLNMSGPFEGG
AVSAASLTQMPAGNVFTTAEGLFSTLPFPVYSNGIHAAQT LERKED

Figure 3

NPAS3 nucleic acid sequence (spliceform incorporating exons 1a-2-3-4etc) similar to mouse cDNA with accession number NM_013780)

```
1  ATGGGGAGGG CCGGCGCCGC GGCCAACGGC ACCCCGCAGA ACGTCCAGGG CATCACCTCC
61  TACCAGCAGC GAATAACTGC CCAGCATCCT CTGCCCCAACC AATCAGAATG TAGGAAAATC
121 TACAGATATG ACGGAATCTA CTGTGAATCT ACCTACCAGA ATTTACAAGC ATTGAGAAAG
181 GAGAAATCCC GAGATGCTGC TCGCTCCCGC CGGGGAAAAG AAAACTTTGA GTTCTATGAA
241 TTGGCCAAGT TGTGCCTCT TCTGCAGCC ATTACCAGCC AGCTCGACAA GGCATCCATC
301 ATTCGACTTA CAATTAGCTA TCTGAAAATG ACGGACTTTG CTAACCAGGG GGACCTCCG
361 TGGAACTTGC GAATGGAAGG CCCTCCACCT AACACATCAG TAAAAGGTGC ACAGCGAAGG
421 AGAAGCCCCA GTGCACTAGC CATTGAAGTA TTTGAAGCAC ATTTGGGAG CCACATTTTG
481 CAGTCCCTGG ATGGCTTTGT ATTTGCACTA AATCAGGAAG GAAAATTTT GTACATTTCC
541 GAAACAGTCT CCATCTACCT AGGCCTCTCA CAAGTGGAGC TGACAGGCAG CAGTGTCTTT
601 GACTATGTCC ACCCCGGAGA TCACGTGGAG ATGGCTGAGC AGCTGGGCAT GAAGCTCCCC
661 CCTGGGCGGG GTCTCCTGTC ACAGGGCACT GCTGAGGACG GAGCCAGCTC AGCATCTTCC
721 TCCTCTCAGT CGGAGACCCC CGAGCCAGTG GAGTCAACCA GCCCAGTCT GCTAACCCT
781 GACAACACTC TTGAGCGTTC CTTTTTCATC CGAATGAAAT CTACTCTGAC CAAACGCGGT
841 GTGCACATCA AATCATCAGG ATATAAGGTG ATTCACATAA CAGGCCGGCT ACGCCTGAGA
901 GTGTCGCTGT CCCACGGGAG GACCGTCCCC AGCCAAATCA TGGGTCTCGT GGTGTGTGCG
961 CATGCCTTGC CTCCCCCTAC GATCAATGAA GTCAGAATTG ACTGCCATAT GTTCGTCCT
1021 CGAGTAAATA TGGACCTCAA TATCATTTAC TGTGAAAATA GGATTAGTGA TTATATGGAT
1081 CTGACCCCTG TAGATATCGT AGGGAAGAGA TGCTACCACT TCATCCATGC TGAAGACGTG
1141 GAGGGCATCA GGCACAGTCA CTTGGACTTG CTGAATAAGG GTCAGTGTGT GACAAAGTAC
1201 TATCGCTGGA TGCAGAAGAA CGGAGGATAT ATTTGGATAC AGTCCAGTGC CACCATAGCT
1261 ATTAATGCCA AGAATGCAAA TGAAAAGAAT ATCATCTGGG TGAATTACCT TCTTAGCAAT
1321 CCTGAGTACA AGGACACACC CATGGACATC GCACAGCTCC CCCATCTGCC GGAGAAAAC
1381 TCCGAATCCT CGGAGACATC CGACTCTGAG TCAGACTCTA AAGACACCTC AGGTATTACA
1441 GAGGACAACG AGAACTCCAA GTCCGACGAG AAGGGGAACC AGTCCGAGAA CAGCGAAGAC
1501 CCGGAGCCCG ACCGGAAGAA GTCGGGCAAC GCGTGTGACA ACGACATGAA CTGCAACGAC
1561 GACGGCCACA GCTCCAGTAA CCCGGACAGC CGCGACAGCG ACGACAGCTT CGAGCACTCG
1621 GACTTTGAGA ACCCAAGGC GGGCGAGGAC GGCTTCGGTG CTCTGGGCGC GATGCAGATC
1681 AAGGTGGAGC GCTACGTGGA GAGCGAGTCG GACCTGCGGC TGCAGAACTC CGAGTCACTC
1741 ACGTCCGACA GCGCCAAGGA CTCGGACAGC GCAGGCGAGG CGGGCGCGCA GGCTCCAGC
1801 AAGCACCAGA AGCGCAAGAA AAGGCGGAAA CGGCAAAAGG GCGGCAGCGC CAGCCGCCGG
1861 CGCCTGTCCA GCGCGTCGAG CCCAGGCGGC CTGGACGCGG GCCTGGTGGA GCGCCGCGG
1921 CTGCTGTCTT CCCCCAACAG TGCCTCGGTG CTCAAGATCA AGACGGAGAT CTCAGAACCC
1981 ATCAATTTTC ACAATGACAG CAGCATCTGG AACTACCCGC CCAACCGGGA GATCTCCAGG
2041 AACGAGTCCC CCTACAGCAT GACCAAGCCC CCCAGCTCTG AGCACTTCCC GTCCCCGAG
2101 GCGGCGGGCG GTGGGGGTGG CGGTGGCGGG GGGCTGCACG TGGCCATTCC CGACTCGGTC
2161 CTCACCCCGC CCGGCGCCGA CGGCGCGGCC GCCCGCAAGA CTCAGTTCCG CGCCTCGGCC
2221 ACCGCGGGCC TGGCCCCCGT CGCCTCCGAC CCGCTGTAC CCCGCTCTC GCGTCCCCG
2281 CGGGACAAGC ACCCGGGGAA CGGCGGCGGG GCGGGGGGCG GGGCGGCGG CGCGGGGGG
2341 GCGGCGCCCA GCGCGTCCAA CTCCTTGCTG TACACTGGGG ACCTGGAGGC GCTGCAGAGG
2401 TTGCAGGCGG GCAACGTCGT GCTCCCGCTG GTGCACAGGG TGACGGGGAC CCTGGCCGCC
2461 ACCAGCACGG CCGCGCAGAG GGTCTACACC ACGGGCACCA TCCGCTACGC GCGCGCCGAG
2521 GTGACCCTGG CCATGCAGAG CAACCTGCTG CCCAACGCGC ACGCTGTAA CTTCTGTGAC
2581 GTTAACAGCC CCGGCTTTGG CCTCGACCCC AAGACGCCCA TGGAGATGCT CTACCACCAC
2641 GTGCACCGGC TCAACATGTC AGGACCGTTC GGCGGCGCAG TGAGCGCAGC TAGCCTGACG
2701 CAGATGCCCC CCGGCAACGT GTTACCACG GCGAGGGGAC TCTTCTCCAC GCTGCCCTTC
2761 CCCGTCTACA GCAACGGCAT CCACGCGGCA CAGACTCTGG AGCGCAAGGA GGACTGAGGC
2821 GCCGCCCGTC CTGGGCCCGG CCAGGCCCGG CTTGGAGGAG GCATCGTCGG CATTTTCGTT
2881 TAGACCTTTA ATTCTAGCAC TTTGAATTCT AGCAGGTCAG CGTCTTCTCT CGCCACGACG
2941 GTCCCCATTC CACCCCTCT T
```

Figure 4

NPAS3 protein sequence of spliceform incorporating exons 1a-2-3-4etc.

LRKEKSRDAARSRRGKENFEFYELAKLLPLPAAITSQLDKASIIRLTISYLKMRDFA
NQGDPPWNLRMEGPPPNTSVKGAQRRRSPSALAIEVFEAHLGSHILQSLDGFVFALN
QEGKFLYISETVSIYLGSLQVELTGSSVFDYVHPGDHVEMAEQLGMKLPPEGRLLSQ
GTAEDGASSASSSSQSETPEPVESTSPSLLTTDNTLERSFFIRMKSTLTGRGVHIKS
SGYKVIHITGRLRLRVSLSHGRTVPSQIMGLVVVAHALPPPTINEVRIDCHMFVTRV
NMDLNIIYCENRISDYMDLTPVDIVGKRCYHFIHAEDVEGIRHSHLDLLNKGQCVTK
YYRWMQKNGGYIWIQSSATIAINAKNANEKNI IWVNYLLSNPEYKDT PMDIAQLPHL
PEKTSESSETSDSESDSKDTSGITEDNENSKSDEKGNQSENSEDPEPDRKKSGNACD
NDMNCNDDGHSSSNPDSRDSDDSFHSDFENPKAGEDGFGALGAMQIKVERYVESES
DLRLQNCESLTSDSAKDSDSAGEAGAQAASSKHQKRKKRRKRQKGGASRRRLSSASS
PGGLDAGLVEPPRLSSPNSASVLKIKTEISEPINFDNDSSIWNYPNREISRNEP
YSMTKPPSSEHFPSPOGGGGGGGGGGGLHVAIPDSVLTPPGADGAAARKTQFGASAT
AALAPVASDPLSPPLSASPRDKHPGNGGGGGGGGGGAGGGGPSASNSLLYTGDLEAL
QRLQAGNVVLPLVHRVTGTLAATSTAAQRVYTTGTIRYAPAEVTLAMQSNLLPNAHA
VNFVDVNSPGFGLDPKTPMEMLYHHVHRLNMSGPFGGAVSAASLTQMPAGNVFTTAE
GLFSTLPFPVYSNGIHAAQTLEKED

Figure 5 Representation of the balanced translocation disrupting the NPAS3 gene on chromosome 14

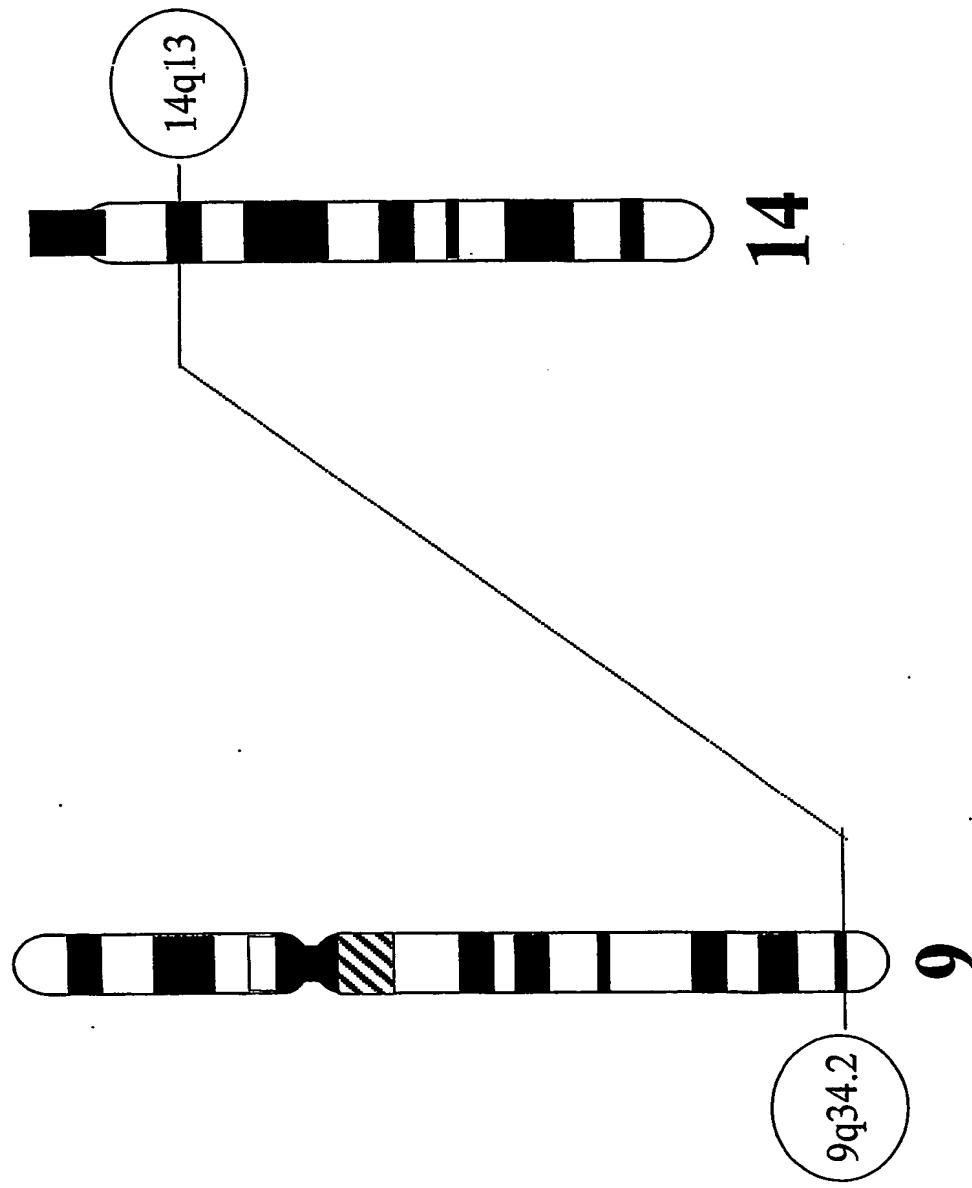
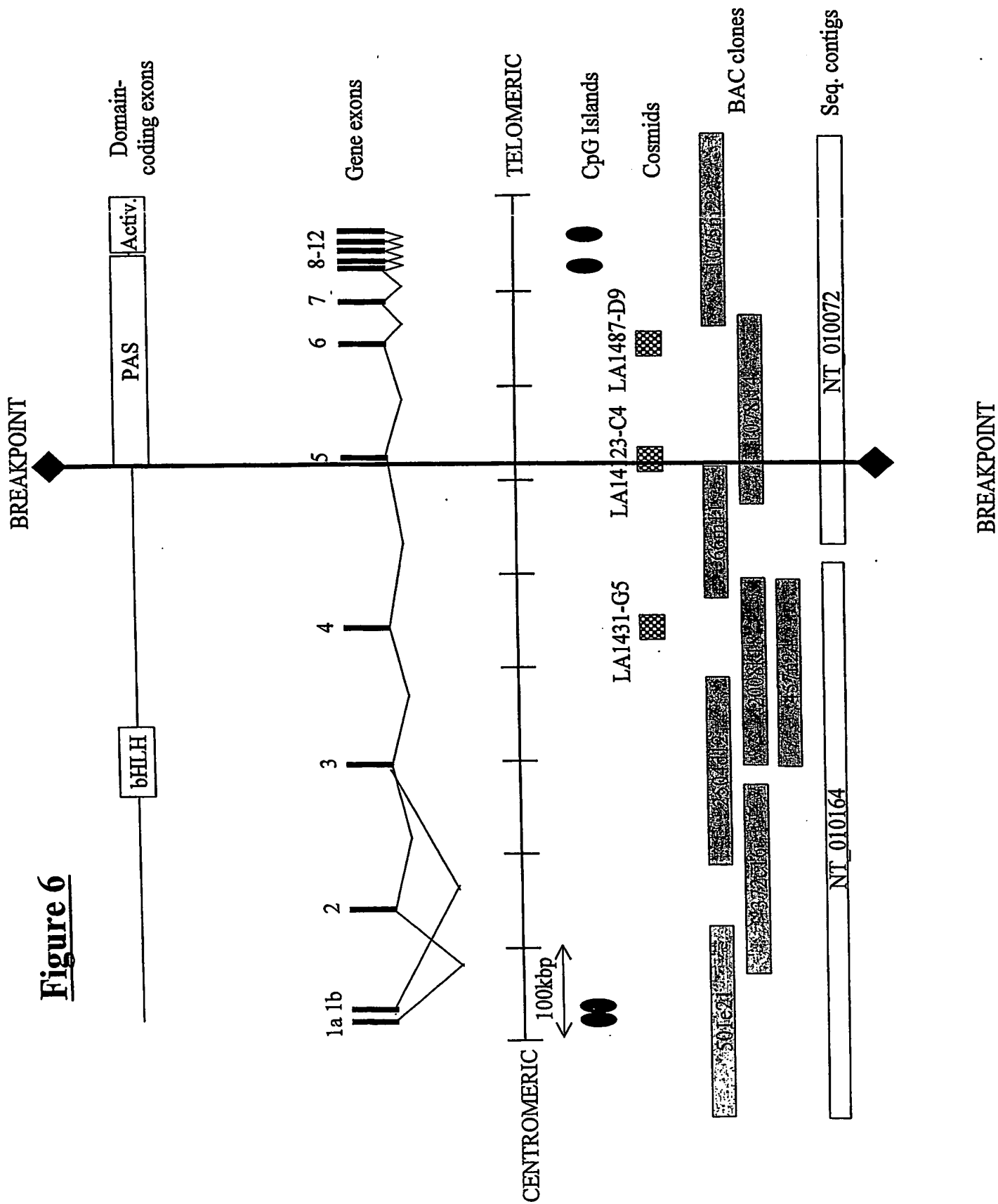


Figure 6



The position of the breakpoint may cause the production of a truncated polypeptide with dominant negative activity

Figure 7

